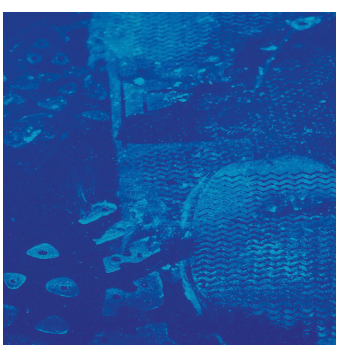
Dycem®

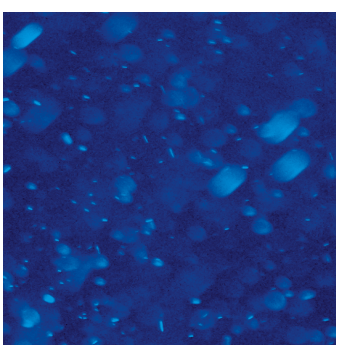
CleanZone Technology

UWE BRISTOL

University of the West of England

Time lapse photography to demonstrate the potential antimicrobial abilities of Dycem contamination control flooring.



TIME LAPSE PHOTOGRAPHY **TO DEMONSTRATE THE POTENTIAL ANTIMICROBIAL ABILITIES OF DYCEM CONTAMINATION CONTROL FLOORING.**

EXECUTIVE SUMMARY

A defined volume of bioluminescent reporter bacteria was deposited onto 20 mm x 20 mm coupons of Dycem flooring, vinyl tile and ITW Alma tacky mat. Through monitoring of bacterial bioluminescence, metabolic inhibition was observed on all three surfaces however the effect occurred more rapidly on Dycem flooring than on vinyl or ITW Alma. Recovery counts after three hours of exposure to the floor surfaces showed between a 65% and 100% decrease in the number of viable organisms recovered from Dycem flooring compared to controls. On vinyl and ITW Alma flooring, there were slight increases in the number of viable survivors recovered compared to controls. Dycem flooring inhibited the metabolic activity and reduced the number of viable survivors recovered of Salmonella enterica Serovar Typhimurium DT104 pGLITE. It remains unclear as to whether this effect is attributable to the impregnated biocide or rapid drying of the inoculum on this surface.

METHODS

Bacterial growth conditions and media. Escherichia coliO157:H7tox-pLITE and Salmonella enterica Serovar Typhimurim DT104 pGLITE were cultured on nutrient agar at 37°C supplemented with 10 mg/L ampicillin or 10 mg/L gentamicin respectively. Antibiotic selection was used to maintain the plasmid-borne lux genes and the bioluminescent phenotype. Overnight cultures were prepared in 10 mL nutrient broth, supplemented with 10 mg/L ampicillin/gentamicin as appropriate and incubated at 37°C in an orbital shaker at 200 rpm.

Preparation of surface materials. Dycem antimicrobial flooring, ITW Alma tacky mat and vinyl tiles were cut into 20 mm x 20 mm coupons. The adhesive surface of ITW Alma tacky mat was revealed immediately prior to inoculation. The test surfaces were clean and dry at the time of inoculation. Where recovery counts were performed, the coupons were sterilised using 100% ethanol followed by air-drying and UV irradiation.

Preparation of inoculum. 100 µL from an overnight culture was subcultured into 55 mL fresh, pre-warmed nutrient broth supplemented with selective antibiotic in a sterile Erlenmeyer flask. The flask was incubated at 37°C in an orbital shaker at 200 rpm until an optical density at 600 nm (OD600) of 0.4-0.6 was achieved. 50 mL of the culture was centrifuged at 4100 rpm for 20 minutes at 20°C. The resultant pellet was resuspended in 50 mL of fresh nutrient broth or sterile Ringer's solution as appropriate, or in a tenfold lower volume of nutrient broth for imaging purposes.

Inoculation. The test materials were carefully inoculated with a defined volume of bacterial suspension (see below). The inoculum was placed on the material surface as a single droplet. For imaging experiments, the test coupons were arranged in a square array left open to the air. For recovery count experiments, sterilised coupons were arranged in sterile petri dishes. Following inoculation the lids were replaced and the plates left undisturbed in a class II microbiological safety cabinet with the fan turned off or on to provide still or moving air around the dish.

Data collection. For imaging experiments, a single 60 second integration was captured every 4 minutes to produce a series of still images over approximately 6.5 hours using an iXonEM+ DU-897BV imaging system (Andor, Belfast, UK). The still images were used to produce the videos appended to the report. Raw data was also analysed to show the decrease in light output over time. For recovery count experiments, the inoculated coupons were transferred to 50 mL Falcon tubes containing 20 mL buffered peptone water supplemented with 10 mg/L selective antibiotic (recovery diluent) and transferred to an orbital shaker shaking at 200 rpm for 20 minutes. The recovery diluent was diluted 1:10 in sterile Ringer's solution and triplicate recovery plates were produced using a Whitley Automatic Spiral Plater

3 (Don Whitley Scientific, Shipley, UK). Plates were incubated overnight at 37°C followed by recovery counts.

Experimental schedule. The material samples were inoculated with the defvolume of bacteria listed below:

- **Imaging experiment 1. E. coli pLITE suspended in nutrient broth and Ringer's solution. 20 µL deposited volume.**
- **Imaging experiment 2. S. enterica sv. Typhimurium pGLITE suspended in nutrient broth and Ringer's solution. 50 µL deposited volume.**
- **Imaging experiment 3. S. enterica sv. Typhimurium pGLITE suspended in nutrient broth. 50 µL deposited volume.**
- **Imaging experiment 4. S. enterica sv. Typhimurium pGLITE suspended in nutrient broth. Inoculum concentrated tenfold. 50 µL deposited volume.**
- **Imaging experiment 5. S. enterica sv. Typhimurium pGLITE suspended in nutrient broth. Inoculum concentrated tenfold. 50 µL deposited volume.**
- **Recovery count experiment 1. S. enterica sv. Typhimurium pGLITE suspended in Ringer's solution. 50 µL deposited volume. 3 hour contact time. Still air.**
- **Recovery count experiment 1. S. enterica sv. Typhimurium pGLITE suspended in Ringer's solution. 50 µL deposited volume. 3 hour contact time. Moving air.**

RESULTS AND DISCUSSION

The initial imaging experiment using E. coli O157 pLITE resulted in images with a poor signal-to-noise ratio which were unsuited to conversion to video format. It was decided to utilise S. enterica sv. Typhimurium pGLITE as it has proven in the past to be a consistently bright reporter strain.

The initial imaging experiment used this organism suspended in nutrient broth and Ringer's solution in order to determine the most suitable medium for subsequent imaging work. The video file and associated stills are located in the VIDEO 1 folder of the appended disc. The flooring samples inoculated with Ringer's solution and nutrient broth are shown to the left and right of centre respectively. Raw data from these images are shown in Figures 1 and 2 for Ringer's solution and nutrient broth respectively. In Ringer's solution, and indeed throughout the experimental run work, there are differences in the initial light output from each floor surface. This is due to the differing colouration of the surfaces. Dycem flooring, being the darkest in colour absorbs incident light whereas the supplied vinyl flooring was very light in colour, reflecting incident light towards the lens. The key factor in assessing the data is not the starting light level, but the rate of decline. All three surfaces tested showed a similar initial decline in light output **(FIGURE 1)** however on Dycem flooring, light levels were reduced to background within approximately 90 minutes. ITW Alma showed a very similar initial drop in light, however the rate of decline slowed and a drop to background was not achieved until approximately 4.5 hours. On vinyl flooring the decline in light was initially rapid, slowing at just after 1 hour and reaching background levels after approximately 4 hours.

FIGURE 1

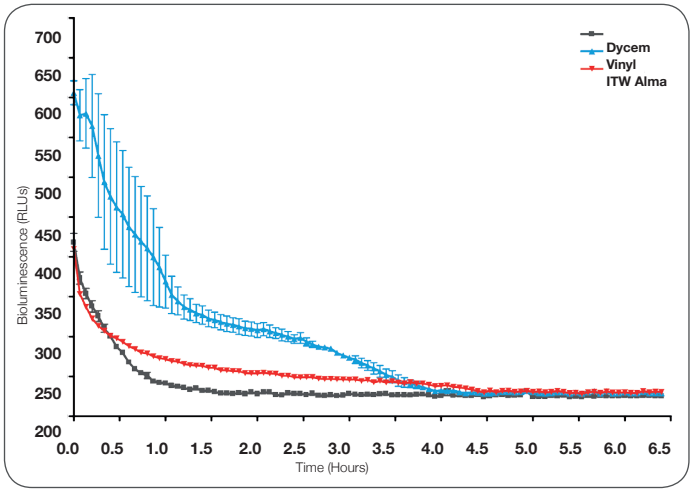
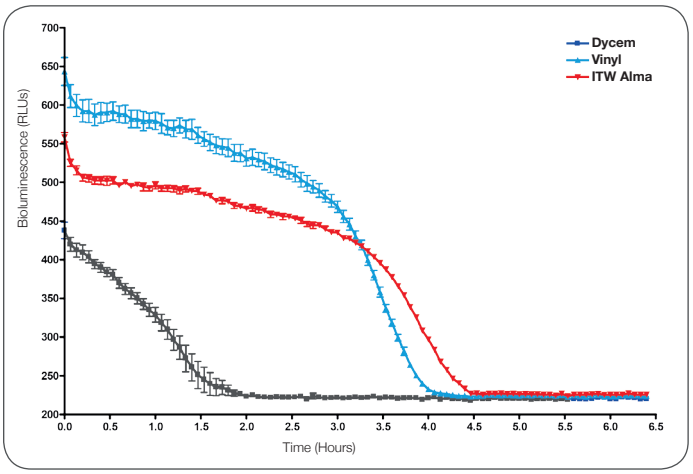


Figure 1: Light output from S.ENTERICA SV typhimurium pGLITE suspended in ringer's solution inoculated onto a range of flooring surfaces. n=3

FIGURE 2



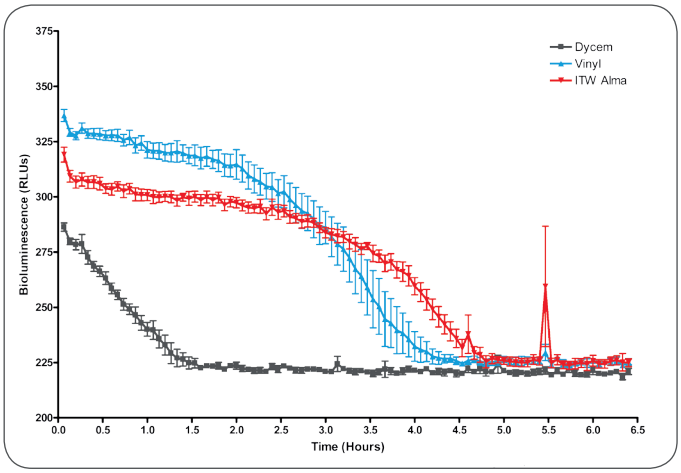
Reduction to background levels took approximately the same time when the inoculum was suspended in nutrient broth **(FIGURE 2)** however light levels remained higher for longer. Light levels on Dycem flooring were reduced to background levels substantially faster than on ITW Alma or vinyl tile, suggesting that metabolic inhibition occurs much more rapidly on Dycem flooring. Prior to the current work commencing it had been observed that the physical nature of Dycem flooring encouraged liquid to spread rather than remain as droplets. Following the completion of imaging, all samples had dried out. VIDEO 1 shows that the droplets placed on Dycem flooring spread before the cessation of light emission whereas on the other two surfaces the droplet remained intact. It is not clear whether the rapid metabolic inhibition on Dycem flooring is due to the impregnated biocide, accelerated drying on this surface, or a combination of both.

Figure 2: Light output from S.ENTERICA SV typhimurium pGLITE suspended in nutrient broth inoculated onto a range of flooring surfaces. n=3

FIGURE 3

Three subsequent imaging experiments were conducted using S. enterica sv. Typhimurium pGLITE in nutrient broth alone as this offered higher light levels than suspension in Ringer's solution. **(FIGURE 3)** shows that the pattern of decline in light output is the same as described above. Inhibition of metabolic activity occurs substantially faster on Dycem flooring that on its competitors and on vinyl tile slightly faster than ITW Alma. VIDEO 2 shows the same rapid spreading of the inoculum followed by a decline in light in Dycem flooring observed previously.

Figure 3: Light output from S.ENTERICA SV typhimurium pGLITE suspended in nutrient broth inoculated onto a range of flooring surfaces. n=3



RESULTS AND DISCUSSION

(FIGURES 4 AND 5) represent the data gathered from VIDEO 3 and VIDEO 4. These were repeats of the previous imaging experiment however the bacterial inoculum had been concentrated tenfold prior to inoculation to further improve the signal-to-noise ratio. The increased inoculum does not affect the time taken for bioluminescence to decrease to background levels suggesting that drying is a factor in the metabolic inhibition observed as the deposited volume was unchanged. Both video files clearly show that metabolic inhibition occurs more rapidly on Dycem flooring than on its competitors.

FIGURE 4

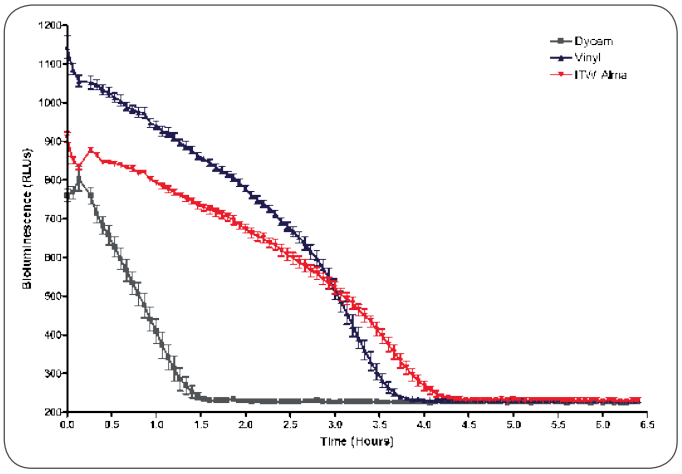


Figure 4: Light output from S.ENTERICA SV typhimurium pGLITE suspended in nutrient broth inoculated onto a range of flooring surfaces. n=3

FIGURE 5

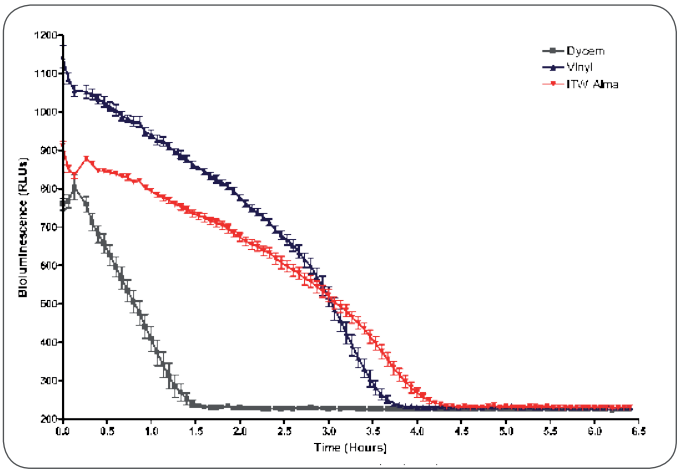
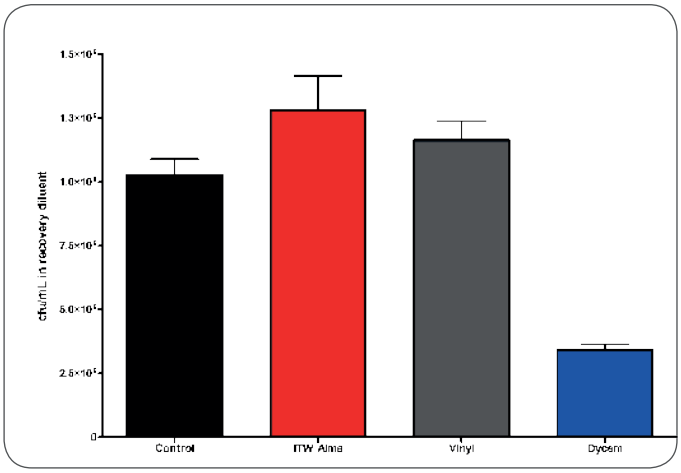


Figure 5: Light output from S.ENTERICA SV typhimurium pGLITE suspended in nutrient broth inoculated onto a range of flooring surfaces. n=3

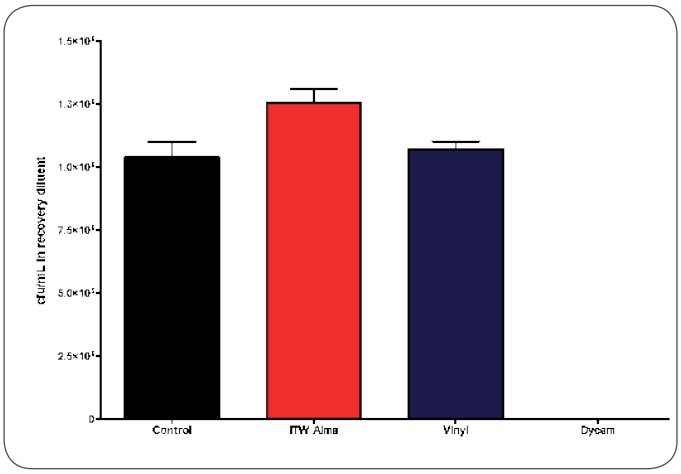
FIGURE 6



(Figures 6 and 7) show the results of recovery counts when S. enterica was inoculated onto the flooring surfaces and left undisturbed for three hours. In both cases, more bacteria were recovered from the vinyl tile and ITW Alma than were present in the control. Since the inoculated samples were covered, there was an element of protection from desiccation not present during the imaging experiment. This may have allowed for limited bacterial growth on the flooring, or growth within the recovery diluent at the end of the contact period. On Dycem flooring however, bacterial numbers were reduced by over 65% **(FIGURE 6)** when left in undisturbed air.

Figure 6: Recovery count from flooring samples inoculated with S. enterica sv Typhimurium pGLITE for a period of three hours in undisturbed air. n=3

FIGURE 7



On these flooring samples, the inoculum had not dried out but it had spread by the end of the experimental period whereas the inoculum on vinyl tile and ITW Alma remained as an intact droplet. Where the class II safety cabinet was operational **(FIGURE 7)**, even though the plates were covered, the inoculum had completely dried on the Dycem flooring. In this instance there were no viable survivors recovered from Dycem flooring. On ITW Alma and on vinyl, numbers of survivors were once again slightly higher than the control.

Figure 7: Recovery count from flooring samples inoculated with S. enterica sv Typhimurium pGLITE for a period of three hours in undisturbed air. n=3

SUMMARY

The experimental work undertaken has shown that metabolic inhibition of S. enterica occurs more rapidly on Dycem flooring than on either vinyl tile or ITW Alma flooring. Recovery counts have shown that fewer viable bacteria were recoverable from Dycem flooring than from vinyl tile or ITW Alma flooring. From the limited nature of the experimental work conducted it is not possible to ascertain whether the inhibitory effect of Dycem flooring is due to the impregnated biocide or the more rapid spreading and drying of liquids on the surface of Dycem.